Isolation of KPC-2-producing Klebsiella pneumoniae strains belonging to the high-risk multiresistant clonal complex 11 (ST437 and ST340) in urban rivers

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References


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Sir,

The dissemination of KPC-producing bacteria is a major clinical and public health concern worldwide. In this regard, this journal recently published a molecular study of representative KPC-2-producing Klebsiella pneumoniae clinical strains that had been isolated in different Brazilian states, which revealed the dissemination of high-risk multiresistant clones belonging to the multilocus sequence typing (MLST) clonal complex (CC) 11, with sequence type (ST) 437, ST340 and ST11 playing an important role. In this study, we report for the first time that the environmental dissemination of KPC-2-producing K. pneumoniae belonging to the high-risk multiresistant CC11 is ongoing.

From January 2011 to June 2011, during a local surveillance study that had been established to monitor the occurrence of carbapenemase-producing Gram-negative bacteria from urban rivers in south-eastern Brazil, three carbapenem-resistant K. pneumoniae isolates were recovered from the Tietê (TIET) and Pinheiros (PINH) rivers. The Tietê is one of the main rivers of the region, which runs across São Paulo state from east to west for about 1100 km, whereas the Pinheiros River is a tributary of the Tietê River that runs 25 km through the city of São Paulo, the largest and most populous metropolitan area in Brazil.

Following standard methods for the examination of water and wastewater, 500 mL of surface water was collected in sterile bottles (http://www.standardmethods.org). From each water sample, 100 mL was concentrated by filtration through sterile membrane filters with a pore size of 0.45 μm. The filters were placed on MacConkey agar plates and incubated for 24 h at 37°C. Next, the membrane filters were aseptically removed and placed separately into sterile tubes that had previously been filled with 10 mL of sterile Mueller – Hinton broth. After vortex treatment, an aliquot (100 μL) of each culture was streaked onto CHROMagar™ KPC (Paris, France).

Carbapenem-resistant K. pneumoniae strains exhibited a multidrug-resistant phenotype (Table 1) and grew efficiently on carbapenemase-producing Gram-negative bacteria from urban rivers in south-eastern Brazil, three carbapenem-resistant K. pneumoniae isolates were recovered from the Tietê (TIET) and Pinheiros (PINH) rivers. The Tietê is one of the main rivers of the region, which runs across São Paulo state from east to west for about 1100 km, whereas the Pinheiros River is a tributary of the Tietê River that runs 25 km through the city of São Paulo, the largest and most populous metropolitan area in Brazil.

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Carbapenem-resistant K. pneumoniae strains exhibited a multidrug-resistant phenotype (Table 1) and grew efficiently on CHROMagar™ KPC, yielding metallic blue colonies. Carbapenemase production was confirmed by a modified Hodge test and an inhibition test with phenylboronic acid. Furthermore, PCR and sequencing revealed that these isolates harboured a KPC-2 gene, located on transposon Tn4401b, and blaCTX-M-16-type extended-spectrum β-lactamase genes (Table 1). The positive water samples were collected from two sites located in the metropolitan region of São Paulo. The first site (PINH-4900) was located downstream of the Tietê River and the second site (PINH-4901) was located downstream of the Pinheiros River.
Table 1. Characteristics of environmental KPC-2-producing *K. pneumoniae* strains, *E. coli* TOP10 (T-171) and *E. coli* TOP10

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Environmental location (latitude/longitude)</th>
<th>MIC (mg/L)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>β-Lactamase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Inc type</th>
<th>MLST ST/CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kp 148, PINH-4900</td>
<td>S 23°31’52”, W 46°44’54”</td>
<td>&gt;32  &gt;32  &gt;256  &gt;256  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  0.75  32  256  1.0</td>
<td>CTX-M-2, CTX-M-15, KPC-2</td>
<td>IncN</td>
<td>437/11</td>
</tr>
<tr>
<td>Kp 171, TIET-4200</td>
<td>S 23°31’11”, W 46°44’47”</td>
<td>&gt;32  &gt;32  &gt;256  &gt;256  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  128  0.5  4.0  1.0</td>
<td>CTX-M-15, KPC-2</td>
<td>IncN</td>
<td>340/11</td>
</tr>
<tr>
<td>Kp 196, TIET-4200</td>
<td>S 23°31’11”, W 46°44’47”</td>
<td>&gt;32  &gt;32  &gt;256  &gt;256  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  0.75  0.5  256  1.0</td>
<td>CTX-M-2, CTX-M-15, KPC-2</td>
<td>IncN</td>
<td>437/11</td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (T-171)</td>
<td></td>
<td>&gt;32  &gt;32  &gt;256  &gt;256  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  &gt;32  0.016  &gt;32  256  0.25  1.0  0.5</td>
<td>CTX-M-15, KPC-2</td>
<td>IncNd</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> TOP10</td>
<td></td>
<td>0.25  0.25  0.75  0.064  0.006  0.06  0.25  0.004  0.094  0.5  0.25  1.0  0.5</td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup>*K. pneumoniae*; PINH, Pinheiros River; TIET, Tietê River; *E. coli* TOP10 (T-171), *E. coli* TOP10 transformed with plasmid DNA from *K. pneumoniae* strain 171.

<sup>b</sup>MIC determined by Etest, agar dilution and/or broth microdilution methods; resistance is indicated in bold. CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; PMB, polymyxin B; FOF, fosfomycin; TGC, tigecycline. The 2013 CLSI breakpoints were used to interpret the MIC results, except for polymyxin B, for which the EUCAST criteria for polymyxin E (resistant >2 mg/L) were applied (http://www.eucast.org/clinical_breakpoints/). KPC-2-positive *K. pneumoniae* isolates were found to be resistant to amoxicillin/clavulanic acid, cefalotin, cefoxitin, nalidixic acid and levofloxacin, as determined by Kirby–Bauer susceptibility testing.<sup>12</sup>


<sup>d</sup>The *bla*KPC-2 genotype of *E. coli* TOP10 (T-171) was associated with the presence of a transferable IncN plasmid of approximately 40 kb.
Pinheiros River (S 23°31'52", W 46°44'54")}, prior to its confluence with the Tietê River, and the second site (TIET-4200) was located downstream of the Tietê River (S 23°31'11", W 46°44'47"), about 2 km away from the first site. The first KPC-2-positive K. pneumoniae strains (isolates 148 and 171) were identified in March 2011 at PINH-4900 and TIET-4200, respectively, denoting early environmental dissemination. These isolates were clonally unrelated by PFGE and enterobacterial repetitive intergenic consensus sequence-based PCR (ERIC-PCR) and belonged to lineages ST437 and ST340, respectively (http://www.pasteur.fr/recherche/genopole/PO/MLST/Kpneumoniae.html). Three months later, a new KPC-2-producing K. pneumoniae isolate (strain 196) was found at TIET-4200. Surprisingly, this isolate was closely related to the former KPC-2-positive K. pneumoniae strain recovered from the Pinheiros River, belonging to ST437 on MLST analysis (Table 1), which suggests an environmental persistence of CC11. In the K. pneumoniae strain 171-ST340, the bla_{KPC-2} genotype was associated with the presence of a transferable IncN plasmid of approximately 40 kb, which was successfully transformed into the TOP10 E. coli recipient strain (Table 1). In fact, an IncN plasmid backbone was found in all three KPC-2-producing strains.

In Brazil, production of the KPC enzyme has become the most prevalent mechanism of acquired resistance to carbapenems among members of the Enterobacteriaceae, constituting a significant clinical problem. In this regard, KPC-2-producing K. pneumoniae seems to have emerged in 2005, in São Paulo city. Since then, KPC-2-producing K. pneumoniae has become endemic in hospital settings, being recurrently associated with outbreaks of nosocomial infection. Currently, the bla_{KPC-2} gene is no longer limited to K. pneumoniae and, indeed, has been identified in a wide range of Gram-negative bacteria. With respect to CTX-M β-lactamases, isolates carrying CTX-M-2 or CTX-M-15 genes have been highly prevalent in South America and worldwide. The emergence of isolates co-producing KPC-2 and CTX-M enzymes suggests that endemic CTX-M-producing lineages have successfully acquired the bla_{KPC-2} gene.

The detection of KPC-2-producing K. pneumoniae strains belonging to CC11 in urban rivers is epidemiological evidence demonstrating that the environmental dissemination of high-risk multiresistant bacteria is ongoing in Brazil. Indeed, the isolation of Pseudomonas aeruginosa co-producing metallo-β-lactamase SPM-1 and 16S rRNA methylase RmtD1 in the Tietê River has also been reported. In this regard, the Tietê River has several reservoirs along its course, which are widely used for agricultural irrigation or to supply drinking water. Unfortunately, the Tietê and Pinheiros Rivers are affected by the direct discharge of anthropogenic pollutants, including domestic sewage and hospital wastewater. Therefore, urban rivers in Brazil could represent a potential source of human colonization and/or infection.

Of particular interest was the identification of strains belonging to CC11. Considering that CC11 KPC-2-producing K. pneumoniae strains are widespread in hospital settings, speculations on a possible link could be raised. KPC-2-producing Enterobacteriaceae (including Klyuyvera spp. and Raoultella spp.) and Aeromonas species have recently been reported in sewage and wastewater samples obtained from hospitals in São Paulo and Rio de Janeiro, which demonstrates the potential of bla_{KPC-2} to spread among Gram-negative bacteria beyond the hospital.

The surveillance of resistance in environmental samples from urban regions needs to be established as a priority, and strategies for the treatment of wastewater urgently need to be adopted in order to inhibit the release of high-risk multiresistant bacteria into the environment.

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Transparency declarations

None to declare.

References

Metronidazole increases the emergence of ciprofloxacin- and amikacin-resistant Pseudomonas aeruginosa by inducing the SOS response

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Sir,

The SOS response is a conserved regulatory network that is induced in response to DNA damage. During this stress response, the RecA protein, bound to single-stranded DNA, stimulates the cleavage of the repressor LexA, thus releasing the transcription of the LexA-controlled genes. SOS response activation promotes the transfer and expression of foreign resistance genes, but also induces error-prone DNA polymerases PolIV and PolV, increasing the cell’s spontaneous mutation frequency. This is of particular concern with respect to Pseudomonas aeruginosa, in which multidrug resistance mainly arises from chromosomal mutations.

P. aeruginosa is a nosocomial opportunistic pathogen that has the distinctive capacity to become resistant to the major classes of antibiotics by the activation of intrinsic resistance mechanisms. For example, significant resistance to β-lactams may arise from stable up-regulation of the intrinsic cephalosporinase AmpC, increased drug efflux or outer membrane impermeability (alteration of porin OprD). Its resistance to aminoglycosides may involve the overproduction of the MexXY-OprM efflux pump. Similarly, fluoroquinolone resistance may be due to mutations in target genes or drug efflux systems.

The three major classes of antibiotics (β-lactams, aminoglycosides and fluoroquinolones) can directly or indirectly provoke the bacterial SOS response, depending on the experimental conditions and the bacterial species tested. Metronidazole at therapeutic concentrations, which have absolutely no bactericidal effect on P. aeruginosa, also activates this bacterial response, both in vitro and in patients. Metronidazole is widely used to treat infections caused by anaerobic bacteria, protozoa and the microaerophilic Helicobacter pylori. Interestingly, Cipriano Souza et al. showed that the previous consumption of metronidazole was an independent risk factor for the acquisition of multidrug-resistant P. aeruginosa by hospitalized patients. Altogether, this led us to hypothesize that the exposure of P. aeruginosa to metronidazole, through the induction of the SOS response, favours the emergence of resistance to β-lactams, aminoglycosides and fluoroquinolones.

We used the reference P. aeruginosa strain PA14, and its derivative in which recA was deleted using the splicing by overlap extension PCR method, as previously described. We quantified the expression of the SOS pathway genes recA and lexA by RT-qPCR after in vitro induction with metronidazole. Under the same experimental conditions, we assessed the frequency of emergence of bacteria resistant to ciprofloxacin, amikacin, ceftazidime and imipenem. Metronidazole concentrations used for SOS response induction experiments (50 mg/L for PA14 and 25 mg/L for PA14 ΔrecA, corresponding to 1/80 the MIC) were in the range of those found in the plasma of treated patients.

In vitro exposure of the reference strain PA14 to a therapeutic concentration of metronidazole triggered the SOS response, as indicated by increased recA and lexA expression (Figure 1a). It also enhanced the frequency of emergence of bacteria resistant to ciprofloxacin and amikacin by 2.8-fold and 15.4-fold, respectively (Figure 1b and c). By contrast, we found that the frequency of emergence of bacteria resistant to ceftazidime and imipenem remained basal after exposure to metronidazole (data not shown). As shown by the results obtained in the recA-deleted PA14 mutant, the effect of metronidazole on the emergence of bacteria resistant to ciprofloxacin and amikacin fully depended on the presence of recA, confirming the role of SOS response induction in the emergence of multidrug resistance (Figure 1a–c).

Metronidazole and related 5-nitroimidazoles are redox-active prodrugs. Bacterial nitroreductases, such as RdxA in H. pylori, catalyse the conversion of metronidazole into mutagenic products that directly interact with DNA bases. This causes DNA helix destabilization and single- and double-strand DNA breakage, which activates the SOS response. The effect of metronidazole in P. aeruginosa in terms of DNA damage remains to be established, but one can speculate that the RdxA homologue in this species (PA14 68560 in the PA14 genome, http://www.pseudomonas.com) could play a similar role in the metabolism of metronidazole, and may explain how this antibiotic triggers the SOS response.

Our data suggest that SOS response induction by metronidazole enhances the emergence of resistant bacteria in vitro. This is alarming, because not only metronidazole but also the major classes of antibiotics (β-lactams, aminoglycosides and fluoroquinolones) can trigger the bacterial SOS response. In addition to the present in vitro data, the suppression of the SOS response has been shown to reduce the emergence of antibiotic-resistant bacteria in vivo. Altogether, this supports the hypothesis that the inhibition of RecA is a plausible therapeutic adjuvant in combined therapy to reduce the capacity to generate antibiotic-resistant mutants.